



(12) **United States Patent**
Kariko et al.

(10) **Patent No.:** **US 11,028,370 B2**
(45) **Date of Patent:** ***Jun. 8, 2021**

- (54) **RNA PREPARATIONS COMPRISING PURIFIED MODIFIED RNA FOR REPROGRAMMING CELLS**
- (71) Applicant: **The Trustees of the University of Pennsylvania**, Philadelphia, PA (US)
- (72) Inventors: **Katalin Kariko**, Rydal, PA (US); **Drew Weissman**, Wynnewood, PA (US); **Gary Dahl**, Madison, WI (US); **Anthony Person**, Madison, WI (US); **Judith Meis**, Fitchburg, WI (US); **Jerome Jendrisak**, Madison, WI (US)
- (*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 110 days.

This patent is subject to a terminal disclaimer.

(2013.01); *A61K 48/0075* (2013.01); *C07K 14/47* (2013.01); *C07K 14/4712* (2013.01); *C07K 14/505* (2013.01); *C12N 9/0075* (2013.01); *C12N 9/22* (2013.01); *C12N 9/78* (2013.01); *C12N 15/117* (2013.01); *C12N 15/85* (2013.01); *C12N 15/87* (2013.01); *C12N 2310/17* (2013.01); *C12N 2310/335* (2013.01); *C12N 2320/30* (2013.01); *C12N 2501/602* (2013.01); *C12N 2501/603* (2013.01); *C12N 2501/604* (2013.01); *C12N 2501/605* (2013.01); *C12N 2501/606* (2013.01); *C12N 2501/608* (2013.01); *C12N 2506/02* (2013.01); *C12Y 114/13039* (2013.01); *C12Y 301/04012* (2013.01); *C12Y 305/04004* (2013.01)

(58) **Field of Classification Search**
CPC C12N 5/0696; C12N 15/87
USPC 435/377; 536/23.1
See application file for complete search history.

- (21) Appl. No.: **15/994,093**
- (22) Filed: **May 31, 2018**
- (65) **Prior Publication Data**
US 2018/0265848 A1 Sep. 20, 2018

(56) **References Cited**
U.S. PATENT DOCUMENTS

5,824,497 A 10/1998 Andrews et al.
7,335,471 B2 2/2008 Guillerez et al.
7,807,815 B2 10/2010 Maclachlan et al.
8,039,214 B2 10/2011 Dahl et al.
8,278,036 B2 10/2012 Kariko et al.
8,329,887 B2 12/2012 Dahl et al.

(Continued)

- Related U.S. Application Data**
- (63) Continuation of application No. 15/160,062, filed on May 20, 2016, now Pat. No. 10,006,007, which is a continuation of application No. 14/801,075, filed on Jul. 16, 2015, now Pat. No. 9,371,511, which is a continuation of application No. 14/644,680, filed on Mar. 11, 2015, now Pat. No. 9,163,213, which is a continuation of application No. 12/962,468, filed on Dec. 7, 2010, now Pat. No. 9,012,219.
- (60) Provisional application No. 61/267,312, filed on Dec. 7, 2009.

FOREIGN PATENT DOCUMENTS

EP 2072618 6/2009
JP 2002-507121 3/2002

(Continued)

- (51) **Int. Cl.**
C07H 21/02 (2006.01)
C07H 21/04 (2006.01)
C12N 5/074 (2010.01)
C12N 15/87 (2006.01)
C12N 15/85 (2006.01)
A61K 38/17 (2006.01)
A61K 38/18 (2006.01)
A61K 38/44 (2006.01)
A61K 38/46 (2006.01)
A61K 38/50 (2006.01)
A61K 48/00 (2006.01)
C07K 14/47 (2006.01)
C07K 14/505 (2006.01)
C12N 9/02 (2006.01)
C12N 9/22 (2006.01)
C12N 9/78 (2006.01)
C12N 15/117 (2010.01)

OTHER PUBLICATIONS

Aasen et al. 2008. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nature Biotech* 26: 1276-84.

(Continued)

Primary Examiner — Valerie E Bertoglio
(74) *Attorney, Agent, or Firm* — Casimir Jones, S.C.; Jason R. Bond

- (52) **U.S. Cl.**
CPC *C12N 5/0696* (2013.01); *A61K 38/1709* (2013.01); *A61K 38/1816* (2013.01); *A61K 38/44* (2013.01); *A61K 38/465* (2013.01); *A61K 38/50* (2013.01); *A61K 48/005*

(57) **ABSTRACT**

The present invention provides compositions and methods for reprogramming somatic cells using purified RNA preparations comprising single-strand mRNA encoding an iPS cell induction factor. The purified RNA preparations are preferably substantially free of RNA contaminant molecules that: i) would activate an immune response in the somatic cells, ii) would decrease expression of the single-stranded mRNA in the somatic cells, and/or iii) active RNA sensors in the somatic cells. In certain embodiments, the purified RNA preparations are substantially free of partial mRNAs, double-stranded RNAs, un-capped RNA molecules, and/or single-stranded run-on mRNAs.

20 Claims, 41 Drawing Sheets
Specification includes a Sequence Listing.